The University of Chicago

THE IRON METABOLISM OF NORMAL YOUNG WOMEN DURING CONSECUTIVE MENSTRUAL CYCLES

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND HOUSEHOLD ADMINISTRATION

1937

By
RUTH M. LEVERTON

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF NUTRITION
Vol. 13, No. 1, January 1937

The University of Chicago

THE IRON METABOLISM OF NORMAL YOUNG WOMEN DURING CONSECUTIVE MENSTRUAL CYCLES

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND HOUSEHOLD ADMINISTRATION

1937

By
RUTH M. LEVERTON

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF NUTRITION
Vol. 13, No. 1, January 1937

R. UNIV.
BIBLIOTHEEK
LEIDEN

Reprinted from THE JOURNAL OF NUTRITION
Vol. 13, No. 1, January, 1937

THE IRON METABOLISM OF NORMAL YOUNG WOMEN DURING CONSECUTIVE MENSTRUAL CYCLES [1]

RUTH M. LEVERTON
Department of Home Economics and Household Administration, The University of Chicago

THREE FIGURES

(Received for publication July 29, 1936)

Information concerning the quantitative metabolism of iron in normal women is meager. A search through the literature yields little in regard to the interrelation of blood formation, menstruation, and iron retention. The difficulties of analysis of iron in biological materials, the small amount of iron metabolized by the human organism, and the variation in the physiological state of the subject as a result of the menstrual process have all been reasons for the scarcity of research in this field.

Two studies of the iron metabolism of normal women over short periods of time have been reported in the last 2 years. Ohlson and Daum ('35) studied the iron exchanges of three healthy young women under normal conditions of living and at intervals over 2½ months. Complete collections were made during six periods which ranged from 5 to 15 days in length and included at least one menstrual period for each subject. The diets contained "liberal amounts of protein, calories and protective elements." The average daily intake of iron for all periods was 13.78 mg. and the average daily excretion

[1] Partial financial aid for this study was received in the form of the Yardley Foundation Fellowship given by the New Jersey State Federation of Women's Clubs.

was 14.95 mg. The losses in the menstrual hemorrhage were 25.68 and 41.90 mg. for two subjects and 18.16 and 32.35 mg. for two different periods for the third subject. The authors report that there was no relationship between iron retention and the losses during menstruation. Because their observations were limited to isolated portions of the menstrual cycles of the subjects rather than including complete ones the metabolic picture in regard to iron exchanges is incomplete.

The same is true of the study of Farrar and Goldhamer ('35). One subject, a dietitian, lived on a diet which furnished 9.1 mg. of iron daily for 41 days. The average daily intake for 17 days during the intramenstrual phase was 8.3 mg. and the excretion 8.2 mg. Approximately 33 cc. of blood were lost in menstruation. The balance was not given for the rest of the experimental time.

A complete study of the iron metabolism of women involves certain difficulties. Because 70% of the iron in the body is present in the blood (Sherman, '33) a complete iron balance must consider in so far as possible all blood losses. These may be due to the bleeding of a cut finger, a nosebleed, drawing venous blood for routine analysis, and most particularly to menstruation. Having included these additional paths of possible loss of iron the question arises in calculating a balance of iron as to how these losses should be considered. It may be normal for a woman to be in negative balance during the few days of menstruation and in positive balance at some later time to compensate for this loss or to prepare for the next loss. The results of a brief iron balance study on a woman might depend therefore on the relation of the time of the study to her menstrual period. However, this variable could be obviated by determining her exchanges of iron over a period of time which would include all the changes related to the menstrual process. Thus a menstrual cycle—the recurring period during which the body, particularly the mucous membrane of the uterus, passes through all the changes associated with menstruation—appears to be the most logical time unit for a complete study of the iron balance of women. Also because of the

recognized variations inherent in daily metabolism, figures for the total balance during a cycle would be more significant than calculations for average daily balances.

In view of these facts the present investigation was planned for the purpose of observing by means of a continuous controlled balance experiment the total iron exchanges of normal women during consecutive menstrual cycles. It is hoped that interpretation of the data obtained will contribute to the knowledge of the iron requirement of women, the degree of the iron losses during menstruation, the time and extent of the storage of iron following these periodic losses, and the hemoglobin and red cell content of the blood during different phases of the menstrual cycle.

EXPERIMENTAL PROCEDURES

The investigation constituted a balance study which involved the weighing and analyzing of all the food eaten by the subjects and the collection and analysis of all the excreta during the experiment. Beginning January 2, 1935, the study extended continuously over a period of 110 days for two of the subjects and 140 days for the other two subjects. Four healthy young college women served as subjects both for this study and the study of calcium and phosphorus metabolism which was being conducted at the same time. Three of them were 21 years old and were carrying an ordinary schedule of college work. The fourth one was 27 years old and had a strenuous schedule of laboratory work in connection with this metabolism experiment. Determinations of basal metabolic rate which were made on all subjects 3 consecutive days at the beginning and at the end of the study showed them to be normal in this respect. The menstrual process was reasonably regular in all the subjects and normal according to the subjective criteria of amount and duration of flow.

Daily records were kept of each subject's weight, taken without clothing and before breakfast, of the number of hours of sleep, of any unusual exercise engaged in, and of definite emotional or physical disturbances during the study. Daily

determinations of hemoglobin and red blood cells were made during the greater part of the study and the results are reported in a separate article (Leverton and Roberts, '36). The amount of venous blood which was taken twice during each menstrual cycle in connection with the calcium and phosphorus study was recorded so that the iron lost in this way might be included in the final iron balances.

Planning the dietary for this prolonged metabolic study presented a real problem. One of the purposes of the experiment was to study normal iron metabolism in several subjects when there was constant intake of this mineral from dietary sources over a long period of time. However, directly opposed to the theoretical attainment of this situation was the necessity of adjusting the caloric intake to the energy requirement of each subject, of satisfying individual food likes, and of offering enough variety to make it possible for the subjects to be content to live on this diet with never a break in routine for as long as 5 months. In addition to this was the knowledge of the notorious lack of uniformity in the composition of foods available on the retail market and the problem of securing and keeping the foods from a uniform source of supply. In an attempt to reconcile these conflicting factors without sacrificing too much from the standpoint of having a constant intake nor from the standpoint of having a long-time study the total time of the experiment was divided arbitrarily into consecutive 5-day periods. Then a single foundation dietary was planned and used for every subject during every 5-day period. By varying the order and combination of the different foods during a period a wide variety in the menus could be furnished but the total intake of the foundation dietary was still almost identical in kind and amount for all subjects for all periods. In addition to the main diet certain foods chosen because of their low mineral content were permitted ad libitum. The psychological value of this practice was found to be immeasurable. A list of the kinds and amounts of foods used is given in table 1. During the fourteenth to the twenty-second periods subject A received a daily supplement of 5 mg. of iron in the

form of ferric ammonium citrate, and as part of the calcium and phosphorus study subjects C and D received 353 Steenbock units of vitamin D from biologically standardized cod liver oil daily.

Every attempt was made to secure foods of uniform composition. The canned goods were bought in case lots and except when extremely impractical the perishable foods were bought from the same source throughout the 5 months. All the food was prepared and served in one of the nutrition

TABLE 1

Individual dietary for each 5-day period

FOODS FOUNDATION DIET	AMOUNT IN GRAMS	FOODS FOUNDATION DIET	AMOUNT IN GRAMS	FOODS AD LIBITUM
Orange juice	200	Lettuce	50	White bread
Orance ice	100	Onions	60	Butter
Tomato juice	200	Peas, canned	70	Jelly
Tomato soup concentrate	70	Potatoes, white	200–400	Apples
Grapefruit	100	Potatoes, canned sweet	100	Salad dressing
Peaches, canned	80	Whole wheat bread	300	Jello
Pineapple, canned	80	Doughnuts	60	Plain cookies
Banana	100	Bacon	20	Candy
String beans, canned	70	Beef, lean	400	Coffee
Beets, canned	70	Salmon	90	Tea
Cabbage	100	Eggs, whole	150	Distilled water
Carrots	100	Milk	2400	

laboratories. Since the plan of this study was to approximate an ordinary situation of food preparation and consumption no attempt was made to protect the food from contamination with iron before it was served. Enamel and aluminum ware utensils were used for cooking and although distilled water was used instead of tap water it was only because the subjects drank different amounts and the tap water was known to vary in its mineral content. However, as soon as the food was served to the subjects a portion was placed in a screw top glass jar and then every precaution was taken to keep this sample from collecting additional iron from the air, utensils or containers. This portion was used for making the daily

food composites which represented one-tenth of the amount of all food eaten by each subject. The five daily composites were combined to form a period composite for each subject and this was analyzed for iron.

After the subjects had been on the experimental diet for 10 days, collection of the excreta was begun. All collections of urine and feces were made directly into glass containers. Carmine was given to mark the beginning of each period. All stools for each subject during each 5-day period were combined into a period fecal composite and aliquot portions of daily urine collections were combined into a period urinary composite. The menses were collected on a standard brand of cellulose pad from a single lot which had been sampled at random for blank analysis.

The food and fecal composites and the collection of each menstrual flow were made into smooth brown digests with sulfuric acid by the method of Stearns ('29) with modifications necessary to make it applicable to material which was to be used for iron analyses. Instead of being boiled with the acid the material and the acid were heated at 60 to 70° C. for 2 weeks at the end of which time a homogeneous emulsion resulted. This could be stored indefinitely. The urine was acidified and stored also.

The difficulties involved in the micro-analysis of iron in biological materials are widely recognized and have received much critical attention of late. No one method seems satisfactory from every standpoint nor gives results of unfailing accuracy at the hands of all workers. In this study iron was determined as the thiocyanate by the method developed by Stugart ('31). This method with careful application and certain adaptations gave admirably accurate results. Space does not permit reporting here the results of the extensive laboratory work and the meticulous routine necessary to establish definitely the adequacy of this method. Every effort was made to prevent the contamination of the samples by iron-laden dust particles. A small protected laboratory of wood and glass was constructed within the larger chemistry laboratory in order to have a place where the samples could be

handled without their accumulating extraneous iron. The method of treatment of the samples consisted in drying and carefully ashing at about 400°C. aliquot portions of the brown digests and of the urine composites, and dissolving the ash in hydrochloric acid. Aliquot portions of the ash solution were then hydrolyzed with acid to change the pyrophosphates to orthophosphates. The iron present was converted to ferric thiocyanate and its amount determined by colorimetric comparison in amyl alcohol solution with a standard solution of iron. At first triplicate determinations were made on each of the ash solutions of three aliquots from each digest but later this number was reduced to duplicate determinations on the ash solution of each of two aliquots from each digest.

For several reasons only total iron was determined in food and excreta in this study in spite of the fact that recent research indicates that iron must exist in the inorganic form to be available for hemoglobin regeneration. Among these was the fact that the most satisfactory method for determining inorganic iron requires the use of individual samples of fresh food material and not composites in which the lack of a homogeneous mixture makes accurate sampling impossible. Daily analysis of individual samples would have been prohibitive in number because of the variety of foods used in each period and the different combinations in which they were prepared. Since the method of preparation can have such a variable effect on the inorganic iron in foods, figures for the average available iron content secured from analysis of a few samples would have been little less than approximations. Moreover, the non-availability of iron in the organic form for hemoglobin building may be due to its incomplete absorption from the intestine (Vahlteich et al., '36). The problem of absorption and re-excretion of iron by the gastro-intestinal tract is not within the realm of a balance experiment and therefore the determination of inorganic or available iron in the feces would be of no more value than one of total iron. Finally, availability has as yet only been demonstrated to apply to the utilization of iron for hemoglobin formation following very

severe anemia and since iron is an essential constituent of animal protoplasm, even though 70% of it exists as hemoglobin, it seemed more pertinent in this study of iron exchanges in normal women to determine total rather than available iron.

RESULTS

The data from this study of iron metabolism will be presented in the following order: 1) the balance between the average daily intake and fecal and urinary excretion of iron for each 5-day period, for each menstrual cycle, and for the entire study; 2) the iron losses in the menses; 3) the balance for each menstrual cycle including the iron lost in the menses; and 4) the actual balance for each cycle and for the entire study when the iron lost through the drawing of venous blood samples and through a nosebleed for one subject is considered.

Balance between food and excreta. The average daily intake, excretion and balance of iron of the four subjects during the consecutive 5-day periods of the metabolism study were calculated from the results of the analysis of the composites of the food, urine and the feces for each period. The results are presented in table 2 expressed in total milligrams of iron per day and in milligrams of iron per kilogram of body weight per day for each subject for each 5-day period. In this table the excretion includes both urinary and fecal iron. Because the daily quantity of iron in the urine was small (0.19, 0.17, 0.29 and 0.19 mg. for subjects A, B, C and D, respectively) and constant it seemed justifiable to add it to the many times larger fecal excretion and thus report only one figure for the excreta. Even though the daily urinary excretion of iron was not great enough to be significant in determining a daily balance such a loss could not be disregarded over a period of several months.

When the average daily amount of iron ingested is compared with the average daily amount excreted in the feces and urine for the entire metabolic period subjects A, B and C were in positive balance and subject D in slight negative balance.

TABLE 2

Average daily intake, excretion and balance of iron of four subjects during consecutive 5-day periods of the metabolism study

SUBJECT	PERIOD	AVERAGE MILLIGRAMS IRON PER DAY			AVERAGE MILLIGRAMS IRON PER KILOGRAM PER DAY		
		Intake	Excretion	Balance	Intake	Excretion	Balance
A	A	9.86	8.11	+1.75	0.166	0.137	+0.029
	B	12.08	12.83	—0.75	0.204	0.216	—0.012
	C	10.56	10.35	+0.21	0.180	0.177	+0.003
	D	10.16	13.15	—2.99	0.176	0.224	—0.048
	E	12.33	8.11	+4.22	0.210	0.138	+0.072
	F	11.36	9.79	+1.57	0.194	0.167	+0.027
	G	11.84	11.95	—0.11	0.200	0.203	—0.003
	H	14.16	12.67	+1.49	0.241	0.216	+0.025
	I	12.48	12.03	+0.45	0.212	0.205	+0.007
	J	14.00	11.47	+2.53	0.238	0.195	+0.043
	K	11.12	11.07	+0.05	0.227	0.187	+0.040
	L	13.60	12.67	+0.93	0.232	0.216	+0.016
	M	13.44	9.63	+3.81	0.230	0.164	+0.066
	N	17.68	19.39	—1.71	0.300	0.331	—0.031
	O	18.16	25.79	—7.63	0.308	0.437	—0.129
	P	18.16	16.83	+1.33	0.308	0.284	+0.024
	Q	17.04	21.59	—4.55	0.288	0.365	—0.077
	R	16.16	8.29	+7.87	0.272	0.140	+0.132
	S	16.56	14.35	+2.21	0.279	0.242	+0.037
	T	17.28	16.52	+0.76	0.290	0.277	+0.013
	U	17.52	16.92	+0.60	0.295	0.285	+0.010
	V	12.54	12.67	—0.13	0.210	0.212	—0.002
	W	11.60	12.63	—1.03	0.193	0.210	—0.017
	X	10.24	7.87	+2.37	0.170	0.132	+0.038
	Y	11.52	11.71	—0.19	0.190	0.198	—0.008
	Z	12.16	7.15	+5.01	0.204	0.120	+0.084
	Average	13.61	12.89	+0.72	0.231	0.220	+0.011
B	A	10.85	2.30	+8.55	0.180	0.037	+0.043
	B	10.64	10.39	+0.25	0.174	0.172	+0.002
	C	10.96	13.77	—2.81	0.178	0.223	—0.045
	D	10.00	12.41	—2.41	0.164	0.203	—0.039
	E	9.84	8.25	+1.59	0.163	0.136	+0.027
	F	12.56	8.81	+3.75	0.209	0.147	+0.062
	G	10.64	9.85	+0.79	0.175	0.163	+0.012
	H	14.56	11.13	+3.43	0.240	0.183	+0.057
	I	12.56	10.01	+2.55	0.208	0.165	+0.043
	J	12.44	12.89	+0.45	0.205	0.211	—0.006
	K	11.20	14.49	—3.29	0.185	0.240	—0.055
	L	12.88	9.53	+3.35	0.213	0.158	+0.055
	M	12.96	9.61	+3.35	0.214	0.159	+0.055
	N	12.00	14.01	—2.01	0.200	0.234	—0.034
	O	11.92	7.37	+4.55	0.198	0.122	+0.076
	P	13.84	10.65	+3.19	0.227	0.175	+0.052
	Q	13.04	11.45	+1.59	0.216	0.189	+0.027
	R	11.12	10.49	+0.63	0.183	0.173	+0.010
	Average	11.87	10.39	+1.48	0.196	0.172	+0.024

TABLE 2—*Continued*

SUBJECT	PERIOD	AVERAGE MILLIGRAMS IRON PER DAY			AVERAGE MILLIGRAMS IRON PER KILOGRAM PER DAY		
		Intake	Excretion	Balance	Intake	Excretion	Balance
C	A	11.12	9.01	+2.11	0.242	0.198	+0.044
	B	10.08	9.49	+0.59	0.222	0.208	+0.014
	C	10.72	8.37	+2.35	0.235	0.185	+0.050
	D	10.48	9.17	+1.31	0.230	0.201	+0.029
	E	10.72	8.35	+2.37	0.234	0.183	+0.051
	F	13.04	10.61	+2.43	0.287	0.232	+0.055
	G	9.44	11.17	—1.73	0.206	0.244	—0.038
	H	11.76	8.93	+2.83	0.255	0.194	+0.061
	I	10.48	9.57	+0.91	0.228	0.208	+0.020
	J	10.03	7.53	+2.50	0.221	0.163	+0.058
	K	9.44	7.57	+1.87	0.202	0.165	+0.037
	L	8.72	10.61	—1.89	0.190	0.231	—0.041
	M	10.16	9.49	+0.67	0.222	0.207	+0.015
	N	10.72	10.21	+0.51	0.232	0.221	+0.011
	O	11.60	11.25	+0.35	0.251	0.243	+0.008
	P	9.60	11.41	—1.81	0.212	0.251	—0.039
	Q	10.64	8.17	+2.37	0.234	0.180	+0.054
	R	8.24	7.73	+1.51	0.182	0.171	+0.011
	S	10.48	9.25	+1.23	0.233	0.205	+0.028
	T	8.48	8.53	—0.05	0.187	0.189	—0.002
	U	10.80	9.01	+1.79	0.238	0.199	+0.039
	V	10.48	10.13	+0.35	0.234	0.226	+0.008
	W	9.84	12.13	—2.29	0.221	0.273	—0.052
	X	7.92	8.69	—0.77	0.176	0.193	—0.017
	Y	8.00	6.85	+1.15	0.184	0.157	+0.027
	Z	7.76	7.89	—0.13	0.183	0.187	—0.004
	Average	10.03	9.32	+0.71	0.221	0.204	+0.017
D	D	10.16	12.43	—2.27	0.186	0.228	—0.042
	E	10.80	14.99	—4.19	0.200	0.277	—0.077
	F	10.88	11.47	—0.59	0.202	0.214	—0.012
	G	9.36	11.15	—1.79	0.173	0.206	—0.033
	H	12.08	11.31	+0.77	0.222	0.208	+0.014
	I	11.28	4.71	+6.57	0.207	0.086	+0.121
	J	11.44	21.15	—9.71	0.209	0.387	—0.178
	K	16.16	11.95	+5.21	0.295	0.219	+0.076
	L	15.04	13.09	+1.95	0.275	0.239	+0.036
	M	12.16	12.89	—0.73	0.222	0.236	—0.014
	N	11.52	13.29	—1.77	0.209	0.241	—0.032
	O	11.76	9.43	+2.33	0.214	0.171	+0.043
	P	11.60	10.69	+0.91	0.211	0.194	+0.017
	Q	11.28	8.39	+2.89	0.206	0.153	+0.053
	R	10.16	11.71	—1.55	0.185	0.213	—0.028
	Average	11.71	11.91	—0.20	0.214	0.218	—0.004

As given in table 2 the average daily intake of iron for subjects A, B, C and D was 13.61, 11.87, 10.03 and 11.71 mg. and the average daily balance was + 0.72, + 1.48, + 0.71 and — 0.20 mg., respectively. These balances represented 5.3, 12.5, 7.1 and 1.7% of the respective intakes.

When the data are expressed in terms of milligrams of iron per kilogram of body weight and only the averages are considered it appears that the highest retention of iron occurred on the lowest intake but when the figures for individual periods are analyzed other facts may be noted. The average daily intakes of iron for subjects A, B, C and D were 0.231, 0.196, 0.221 and 0.214 mg., respectively, with corresponding balances of 0.011, 0.024, 0.017 and — 0.004. In figure 1 the average daily intake of iron from food per kilogram for each subject during each period is plotted in relation to the average daily retention per kilogram which resulted on each intake. Here it may be seen that in general whether considering all subjects for all periods or each subject for all periods the greater retentions occurred on the greater intakes. Of the twenty-six periods during which the subjects were in negative balance, thirteen occurred when the intake was less than 0.200 mg. per kilogram and the other thirteen cases when the intake was between 0.200 and 0.225 mg. per kilogram. Also during the fifty-two periods when the daily intake was less than 0.225 mg. a negative balance occurred in 26 or 50% of the periods. In other words, in this study there was an even chance of the body being in positive or in negative iron balance when the intake ranged from 0.160 to 0.225 mg. per kilogram, but it is significant to note that no negative balances occurred when the intake was 0.225 mg. or more per kilogram per day.

Observation of these data shows the occurrence of many variations in both intake and output of iron. The extent and significance of these variations are shown in table 3. As might be expected the variability of the iron in the food is much less than that in the excreta. The standard deviations for iron intakes range from 1.20 to 1.65 with an average of 1.35; those for iron excretions from 1.30 to 4.34 with an average of

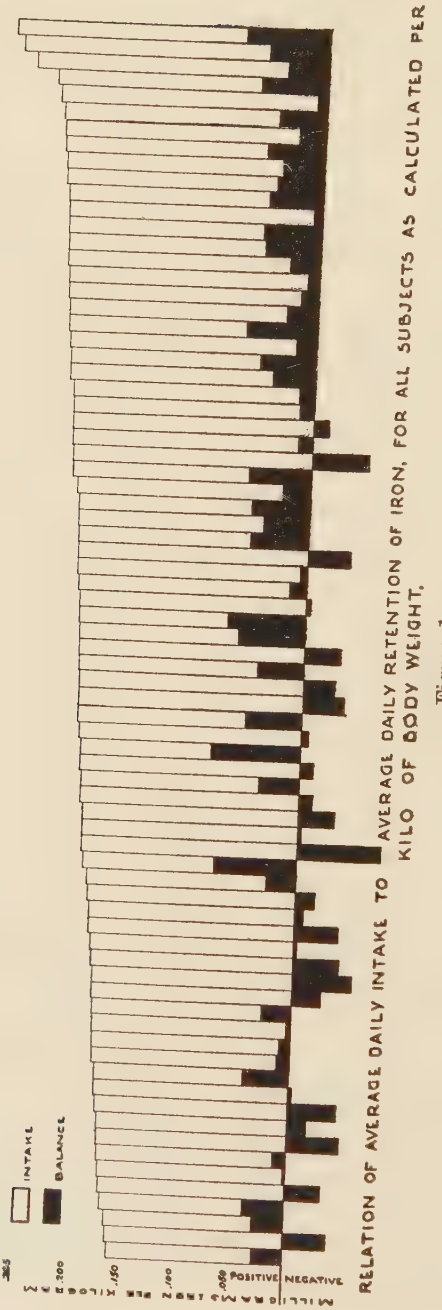
RELATION OF AVERAGE DAILY INTAKE TO AVERAGE DAILY RETENTION OF IRON, FOR ALL SUBJECTS AS CALCULATED PER KILO OF BODY WEIGHT.
INTAKE
BALANCE
MILLIGRAMS IRON PER KILOGRAM
.200
.150
.100
.050
POSITIVE
NEGATIVE

Figure 1

2.96. The average coefficients of variation are 11.9 and 25.8 for iron of food and excreta respectively. For three subjects the variability of the output is at least twice that of the intake and for the fourth subject the two are practically the same.

The variabiliy of the daily balances is best demonstrated by figure 2 which shows graphically the average daily balance of each subject for each 5-day period as given in table 2, for each 10-day period, and for each menstrual cycle. There are periods of negative and positive iron balance for each of the

TABLE 3

Statistical constants

SUBJECT	MEAN	RANGE		STANDARD DEVIATION	COEFFICIENT OF VARIATION
		Lowest	Highest		
Average daily food intake					
	mg.	*mg.*	*mg.*	*mg.*	
Subject A	11.93[1]	9.86	14.16	1.20	10.1
Subject B	11.87	9.84	14.56	1.24	10.5
Subject C	10.03	7.76	13.04	1.30	13.0
Subject D	11.71	9.36	16.16	1.65	14.1
Average daily total excretion					
Subject A	12.89	7.15	25.79	4.34	33.6
Subject B	10.39	2.30	14.49	2.94	28.3
Subject C	9.32	6.85	12.13	1.30	13.9
Subject D	11.90	4.71	21.15	3.27	27.4

[1] Does not include iron from ferric ammonium citrate.

subjects during the metabolism study but no consistent regularity in the order of their occurrence can be detected. Of particular note is the independence of retention with respect to the menstrual periods. The results indicate no regularity in the way the body compensated for the menstrual losses of the subjects.

Effect of ferric ammonium citrate supplement. Beginning with the fourteenth consecutive 5-day period and continuing through the twenty-second period subject A was given a daily supplement of 5 mg. of iron in the form of ferric ammonium

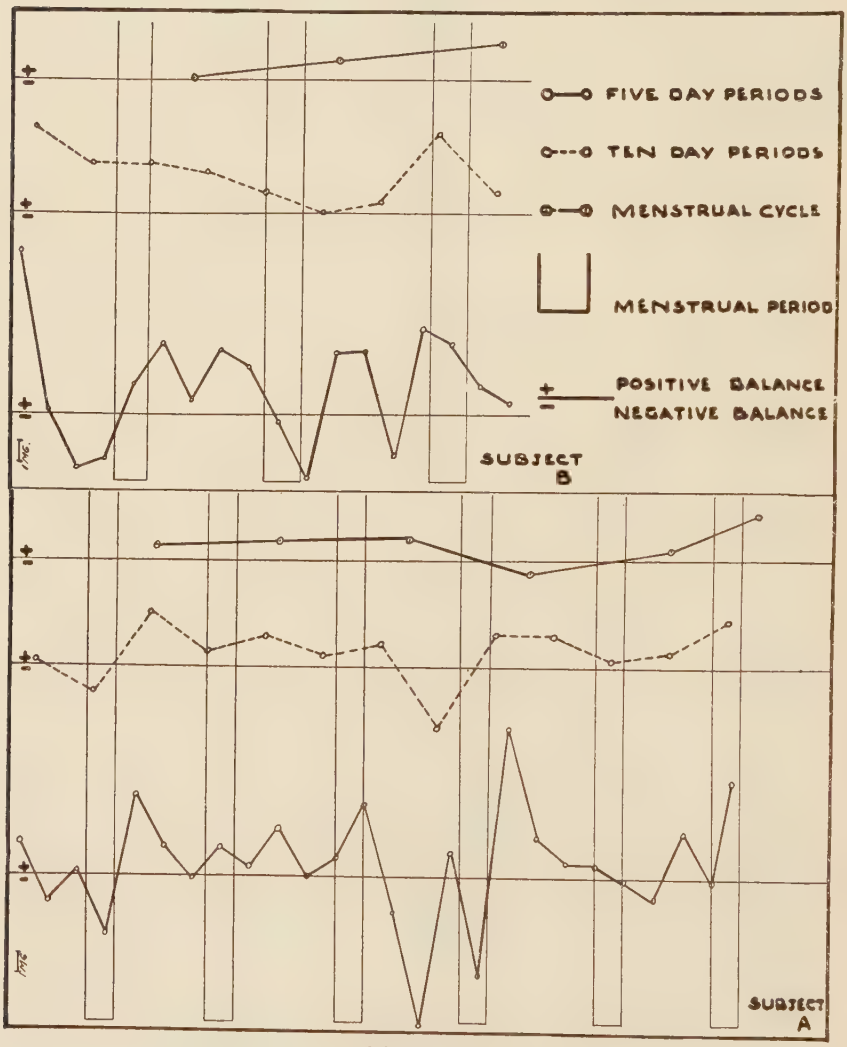
AVERAGE DAILY BALANCE OF IRON
FOR EACH SUBJECT FOR EACH FIVE-DAY PERIOD,
EACH TEN-DAY PERIOD AND EACH MENSTRUAL CYCLE.
FIVE DAY PERIODS
TEN DAY PERIODS
MENSTRUAL CYCLE
MENSTRUAL PERIOD
POSITIVE BALANCE
NEGATIVE BALANCE
SUBJECT B
SUBJECT A

Figure 2

citrate. The effect of this is shown in table 4 where the average daily intake, excretion and balance of iron for the periods before and after the iron therapy are compared with those

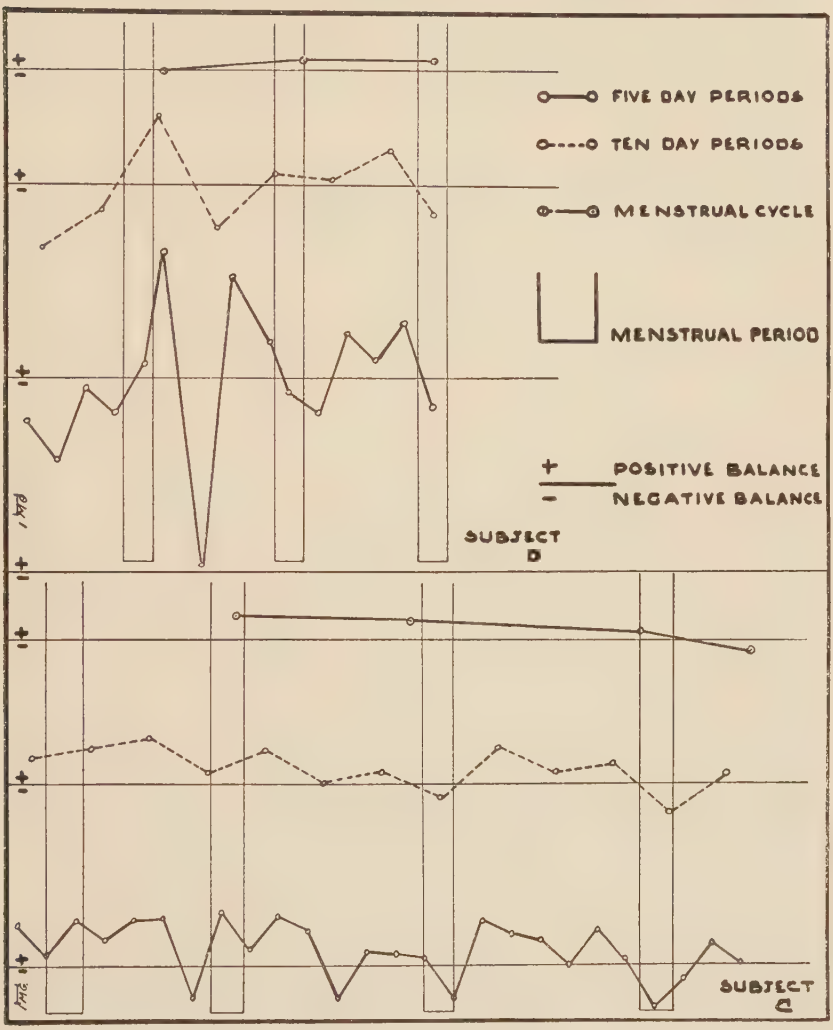

Figure 2—*Continued*

for the periods of iron therapy. During the first thirteen periods of the study subject A had an average positive balance between food and feces and urine of + 1.01 mg. of iron per

day on an average intake of 12.07, but when the average intake was increased to 16.79 by the addition of a solution of ferric ammonium citrate this balance was reduced to — 0.10 mg. per day. When this iron therapy was discontinued and the average daily intake dropped to 11.38 mg. storage took place at the average rate of 1.54 mg. per day. These results were unexpected and a possible explanation will be considered in the discussion.

Menstrual losses. A complete record of the menstrual losses of each subject during each menstrual period throughout the study is given in table 5. Using the figures for the subject's

TABLE 4

Effect of a daily supplement of ferric ammonium citrate on the iron exchange of one subject

PERIODS	TOTAL DAYS	TOTAL PER 5-DAY PERIOD			DAILY AVERAGE			PER KILOGRAM PER DAY		
		Intake	Excr.	Bal.	Intake	Excr.	Bal.	Intake	Excr.	Bal.
A-M inc.[1]	65	60.37	55.32	+5.05	12.07	11.06	+1.01	0.208	0.188	+0.020
N-V inc.[2]	45	83.95	84.47	—0.52	16.79	16.89	—0.10	0.283	0.290	—0.007
W-Z inc.[1]	20	56.90	49.20	+7.70	11.38	9.84	+1.54	0.189	0.165	+0.024

[1] No supplement.

[2] Five milligrams iron daily as ferric ammonium citrate.

own hemoglobin content in grams per 100 cc. it was possible to calculate the volume of blood represented by the iron loss for each subject for each period. Since not all the iron in the blood is in the hemoglobin molecule it is not strictly accurate to convert milligrams of iron into cubic centimeters of blood on the basis of hemoglobin content. However, for practical purposes of expression it is justified because it gives a more understandable measure of the actual menstrual loss and any discrepancies of the procedure are probably within the experimental error of the methods. Both milligrams of iron and cubic centimeters of blood are also calculated on the basis of body weight. Subjects A, B, C and D lost an average of 14.26, 22.84, 11.13 and 13.80 mg. of iron, or 30.01, 50.78, 26.48

and 29.68 cc. of blood, respectively, during each menstrual period. The most striking fact shown by the individual figures is the constant amount of iron lost in the different menstrual periods of the same subject. The variation from one period to the next is less than 10% in the periods of subjects B, C and D though greater for subject A. There appears to be no definite relationship between the iron lost in the menses and

TABLE 5

Menstrual losses of four subjects

SUBJECT	PERIOD	DURATION IN DAYS	TOTAL LOSS		LOSS PER KILOGRAM	
			Milligrams iron	Cubic centimeters blood	Milligrams iron	Cubic centimeters blood
A	I	5	10.46	23.75	0.178	0.403
	II	5	16.80	35.91	0.286	0.611
	III	6	16.40	35.05	0.279	0.597
	IV	5	11.86	23.57	0.201	0.399
	V	6	12.96	25.89	0.217	0.434
	VI	5	17.10	35.92	0.288	0.605
	Average		14.26	30.01	0.241	0.510
B	I	7	22.01	51.62	0.361	0.848
	II	7	22.37	49.73	0.369	0.821
	III	6	24.13	50.98	0.399	0.843
	Average		22.84	50.78	0.380	0.837
C	I	8	10.86	27.49	0.238	0.603
	II	7	11.26	26.98	0.245	0.587
	III	6	10.86	25.29	0.240	0.558
	IV	6	11.56	26.15	0.264	0.597
	Average		11.13	26.48	0.247	0.586
D	I	5	13.26	29.86	0.245	0.551
	II	5	14.52	31.17	0.266	0.570
	III	4	13.62	28.01	0.248	0.510
	Average		13.80	29.68	0.253	0.544

the duration of the period, or to the length of the cycle or to the body weight of the subject.

Another blood loss must be recorded for subject A who had a severe nosebleed the night preceding her fifth menstrual period. The blood was collected on cellulose pads and treated in the same way as the menses. The iron content of the blood loss was 36.57 mg. which was equivalent to about 73 cc. of

her blood. She also had had slight nosebleeds during menstrual periods II and III but because the loss was small the blood was added to the menses for those periods. After the severe loss preceding period V her nostril was cauterized and there was no recurrence of this bleeding.

TABLE 6

Balance of iron for each menstrual cycle for each subject

CYCLE	NUMBER OF DAYS	AVERAGE DAILY			BALANCE FOR CYCLE	LOSS IN MENSTRUATION	FINAL BALANCE	LOSS IN VENOUS BLOOD	ACTUAL BALANCE
		Intake	Excretion	Balance					
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Subject A									
I	22	11.22	10.91	+0.31	+6.72	10.46	—3.74	9.69	—13.43
II	21	12.66	11.68	+0.98	+20.49	16.80	+3.69	10.98	—7.29
III	23	13.96	12.97	+0.99	+22.99	16.40	+6.59	4.68	+1.91
IV	21	17.34	17.95	—0.61	—12.69	11.86	—24.55	11.07	—35.62
V	24	15.04	14.57	+0.47	+11.44	12.96	—1.52	36.57[1] 5.01	—43.10
VI	15	11.31	8.91	+2.40	+36.00	17.10	+18.90	4.76	+14.14
	Total				+84.95	—85.58	—0.63	—82.76	—83.39
Subject B									
I	29	10.79	10.64	+0.15	+4.24	22.01	—17.77	9.38	—27.15
II	27	12.57	11.48	+1.09	+29.53	22.37	+7.16	12.15	—4.99
III	28	12.52	10.55	+1.97	+55.24	24.13	+31.11	0.0	+31.11
	Total				+89.01	—68.51	+20.50	21.53	—1.03
Subject C									
I	30	10.80	9.50	+1.30	+39.06	10.86	+28.20	5.93	+22.27
II	35	10.18	9.20	+0.98	+34.49	11.26	+23.23	12.52	+10.71
III	39	10.00	9.52	+0.48	+18.85	10.86	+7.99	9.59	—1.60
IV	20	8.38	8.89	—0.51	—10.20	11.56	—21.76	6.63	—28.39
	Total				+82.20	—44.54	+37.66	34.67	+2.99
Subject D									
I	27	10.72	10.84	—0.12	—3.25	13.26	—16.51	4.44	—20.95
II	26	13.17	12.70	+0.47	+12.22	14.52	—2.30	9.32	—11.62
III	25	11.26	10.70	+0.56	+14.05	13.62	+0.43	9.72	—9.29
	Total				+23.02	—41.40	—18.38	23.48	—41.86

[1] Loss in nosebleed.

Balances for complete menstrual cycles. In order to give the most complete picture of the iron exchanges in the subjects the data are presented for each menstrual cycle, the logical unit of time for which to calculate an iron balance. In table 6

the average daily intake, excretion and balance of iron is calculated for each cycle and then the total balance for each cycle is presented together with loss in the menses and the balance between these is shown. This is designated as the 'final' balance for each subject, since it is the figure which would be of chief consideration in the usual iron metabolism of women. Since the loss in the venous blood samples and the loss by subject A in a severe nosebleed cannot be disregarded they have been subtracted from the final balance figures and the result designated as the 'actual' balance for the subjects of this study.

It may be noted from the figures given in table 6 that during thirteen of the sixteen menstrual cycles studied the average daily intake exceeded the average daily excretion. The average daily positive balances range from 0.31 mg. to 2.40 mg. with a mean value of 0.93 mg. for all subjects. During the other three cycles there were daily negative iron balances of 0.61, 0.51 and 0.12 mg. between intake and excreta.

The subjects did not always retain enough iron from their intakes to compensate for that lost in the menses. During only nine of the sixteen cycles studied was the total positive balance between intake and excretion sufficient to cover completely the iron lost in the menstrual flow and thus place the subjects in final iron equilibrium or in positive balance. In three other cycles the final negative balances were probably small enough to be negligible, 3.74, 1.52 and 2.30 mg. The final balances for the remaining four cycles were — 16.51, — 17.77, — 21.76 and — 24.55 mg.

Calculation of the actual iron balance of these subjects includes the loss of iron due to the taking of blood samples as well as that in the feces, urine and menses. In only six of the sixteen menstrual cycles studied were the subjects able to compensate for the additional iron lost from the body in the venous blood samples. When the entire time of the study is considered only subjects B and C were near actual iron equilibrium. Subject B accomplished this at one fell swoop in the last cycle by retaining 31.11 mg. which practically compensated for the 32.14 mg. lost in the two previous cycles.

Conversely subject C stored 32.98 mg. in cycles I and II and lost 29.99 mg. in cycle III and the first 20 days in cycle IV. Subject A retained enough to balance the loss in the venous blood samples only during cycles III and VI and subject D never did this. Subject A was in actual negative iron balance to the extent of 83.39 mg. and subject D of 41.86 mg. for the entire study.

Total metabolic picture for the entire study. Since much of the value of this study of iron metabolism lies in the fact that it was continuous over several months a presentation of the complete metabolic picture is important. In figure 3 the total number of milligrams of iron in the food are compared with the total loss of iron in the feces, urine and venous blood samples for each subject for the entire time of the experiment. Under ordinary conditions the final balances between the iron intake and the sum of the iron losses in the feces, urine and menses would give the complete picture of the measurable iron metabolism. Considering just these pathways of loss subjects B and C were in slight positive balance, subject A was in equilibrium, and subject D in negative balance during the entire study. However, the subjects in this study lost additional iron due to the taking of venous blood samples for calcium and phosphorus analysis. Thus when this is considered the 'actual' balances show that subjects B and C were in equilibrium and subjects A and D were in negative balance.

The intakes and losses are calculated also on the basis of milligrams of iron per kilogram per day and shown also in figure 3. By thus ruling out differences due to the varied length of time on the balance study and to unequal body weights the results for all the subjects are comparable. The sum of the losses in the feces, urine and menses for subjects A (omitting the periods of iron therapy), B, C and D were 0.201, 0.192, 0.213 and 0.219 mg. of iron per kilogram per day, respectively. The average is 0.206 mg. These figures will be used later in the calculations of minimum requirement of iron for these subjects and for other normal women.

Hemoglobin and red cell content of the blood. The results of the daily determinations of hemoglobin and red cell content have been reported in a separate article (Leverton and Roberts, '36). Analysis of the data shows the occurrence of daily variations in both hemoglobin and red cells, the majority of which are not greater than the experimental error. The standard deviation for the entire series is 0.90 for hemoglobin and 0.31 for red cells. There was a definite upward trend in the hemoglobin value of every subject during the entire period.

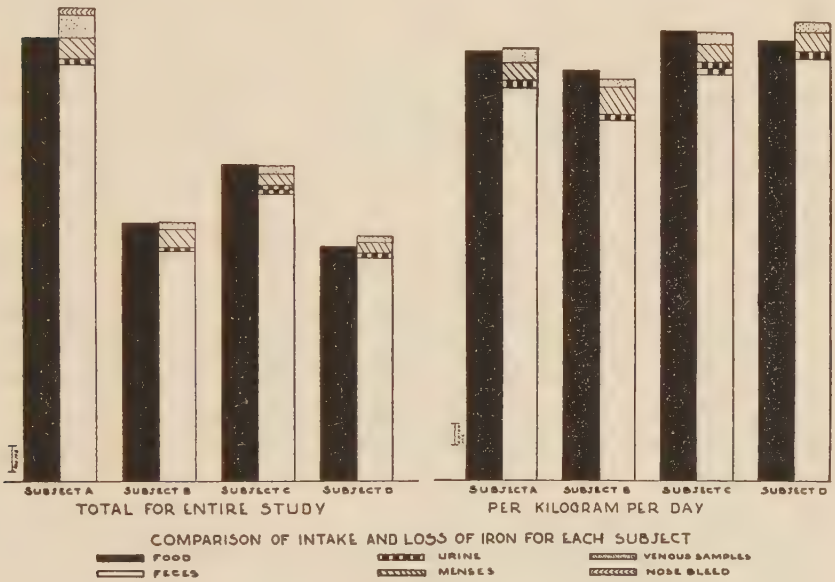

Figure 3

The values increased from 12.95, 12.54, 11.62 and 13.06 gm. per 100 cc. for subjects A, B, C and D, respectively, for the first menstrual cycle to 14.00, 13.92, 13.00 and 14.30 for the last cycle in the study. The red cell count remained remarkably constant for all subjects throughout the experiment. In subject A when 5 mg. of iron from ferric ammonium citrate was given daily during two menstrual cycles the red cell content increased 0.5 million per cubic millimeter and the hemoglobin content increased 1 gm. per 100 cc. These higher

values were not maintained when the citrate was discontinued. From the data presented it appears that there is no measurable or consistent effect of the process of menstruation upon the daily values of either hemoglobin or red cells in the subjects studied. Although occasional marked daily variations in the blood values occur they do so irrespective of the different phases of the menstrual cycle.

A comparison of the average daily iron balance of each subject with the average hemoglobin content of her blood for each 5-day period shows that no measurable relationship between these two factors exists. In many cases the hemoglobin value rises during or following the time when the body is in negative balance or vice versa and a positive balance does not necessarily parallel hemoglobin formation. That the measurement of these two values is not comparable will be emphasized in the discussion.

DISCUSSION

The foregoing data show variations in the iron metabolism of normal young women who had only the demands of maintenance, and were under a controlled dietary regime. Because these variations occur at random during the experimental period of several months they indicate the limitations of the interpretation of results of a metabolism period which is only a few days in length.

Some of the variations observed in this study may be due to fluctuations in the iron content of the diet. Despite precautions taken to control this factor, differences in the iron content of foods due to manufacture, preservation and cooking made some variation inevitable. Also in the present study it was advisable to permit some foods ad libitum and although chosen carefully they were not iron-free and so caused some of the variation observed in the iron intakes of the subjects during the different periods. Marked fluctuation in the iron content of supposedly constant diets, and of samples of the same food taken at different times from different sources, have been reported also by Coons ('35), Toscani and Reznikoff

('34), and Davidson and LeClerc ('36). Such findings together with those of the present study indicate that analysis of each diet as eaten by each subject is the only accurate procedure for balance experiments.

Even though variations occur in the iron intakes they are not as great as the irregularities in the iron excretions and thus in the calculated balances. To what extent the rate of intestinal motility influenced the excretion of iron is speculative. All the subjects had reasonably regular habits of elimination but they did not have the same rhythm or rate of defecation. Subjects A and B had an average of three bowel movements during each 5-day period, and the carmine did not appear in their stools until the second or third morning after it was ingested. The coefficient of variation of the iron content of the excreta in these cases was 38 and 28, respectively. Subject C had two stools a day and the carmine appeared usually within 12 and always within 24 hours after it was taken. In this case the coefficient of variation was only 13, the same as for her intake. Subject D had one movement a day and the carmine always appeared the first morning after it was taken, but even with this regularity the coefficient of variation was 27. It is of interest that subjects A (omitting the periods of iron therapy) and B with a slower intestinal motility had an average daily excretion of 0.177 and 0.172 mg. of iron per kilogram for the entire study and subjects C and D with a more rapid motility had an average daily excretion of 0.204 and 0.214 per kilogram, 18% higher than for A and B. No relationship was found to exist between variations in the fiber content of the diet—entailed by the fact that apples were permitted ad libitum—and variations in the iron excretion.

Evidence of the influence of variations on the interpretation of the results of a metabolism study is demonstrated best by figure 2. In this average total daily retentions are charted for periods of different lengths—5 days, 10 days, and complete menstrual cycles. Careful study of the chart shows that the results from an isolated 5-day period or even a 10-day period would give a very different picture of the iron metabolism of these women than do the results of a continuous study

over a longer period of time. These variations in iron balances when the subjects were under reasonably controlled conditions for several months together with the fact that the variations did not lessen as the study proceeded offer striking evidence of the need for long and continuous metabolism periods if a true record of the body's activity is to be obtained.

It is of interest to compare the average menstrual losses of iron for the subjects in this study with those reported in the literature. Barer and Fowler ('36) have studied the losses of 100 normal individuals during menstruation and found an average iron loss of 19.54 mg. per period. When translated into blood losses on the basis of individual hemoglobin content the average was 50.55 cc. and the standard deviation 25.73 cc. Fifty per cent of her subjects lost between 23.21 and 68.43 cc. of blood in a period. The losses in the present study were 11, 13, 14 and 22 mg. of iron, which represented blood losses ranging from 24 to 52 cc. with an average of 34 cc. Barer's figures and those from this study show that when the amount of blood lost in the menstrual flow is calculated from actual analysis the figures are markedly lower than the usual estimates of 150 cc. to 600 cc. given in physiology textbooks.[2] This increasing knowledge that the normal menstrual losses are probably much less than were heretofore thought and therefore less alarming from the standpoint of the total quantity of blood lost is indeed acceptable. The very constant losses during different periods for the same subject in this study might suggest that in normal women on an adequate regular diet the menstrual loss is definite and characteristic for each individual. This, however, would not be concluded from the findings of Barer who reports marked variation from period to period and more variations for the subjects whose menstrual losses were greater than the average.

The results of increasing the iron intake of subject A by the addition of ferric ammonium citrate were unexpected since reports in the literature indicate that this form of iron is

[2] It has been suggested that gynecologists are prone to disregard these figures and accept the Hoppe-Seyler figure of 37 cc. as nearer the true value.

readily available to the body. The average daily negative balance of 0.10 mg. between food and feces and urine during the periods of increased iron intake is hardly large enough to be significant, but the change in daily iron retention from + 1.01 to — 0.10 when it was added and then from — 0.10 to + 1.54 when it was withdrawn is striking. Although the astringent effect on the intestinal tract of some iron salts is known it has not been reported in the case of ferric ammonium citrate and was not expected of the 3 cc. of 0.95% solution given daily in this case.

The concurrent hemoglobin values for subject A during this time are not in accord with her condition of negative iron balance or even with a condition of equilibrium. During the cycles when she was receiving additional iron her hemoglobin increased from 13.8 to 14.8 gm. per 100 cc. of blood and then dropped to 14.0 when the supplement was withdrawn. The statistical significance of this increase has been considered in the original report (Leverton and Roberts, '36). Hemoglobin formation requires iron, but at no time during the entire study had subject A retained enough from either food or food plus supplement to account for the iron storage represented by the increase in the total hemoglobin content of her blood. This means that the hemoglobin was formed at the expense of the bodily stores of iron and that these stores were not replenished during the experimental regime. Because of the recognized role of copper in hemoglobin formation, it was suspected that the contamination of the ferric ammonium citrate by copper might be one cause for the rise in hemoglobin value. This possibility was precluded, however, when the copper content of the 5-day foundation dietary was calculated and found to be as high as 6 mg. Schlutz, Morse and Oldham ('33) and Fowler and Barer ('35) report large iron retentions and the latter coincident hemoglobin regeneration following the administration of ferric ammonium citrate. The differences between their results and the results of this supplementary iron feeding experiment may be due to several factors. Fowler and Barer used as their subjects six patients with

hypochromic anemia. Although they do not state the amount of the supplement given they report a storage as high as 6.27 gm. of iron in 24 days which would indicate a daily dose of over 250 mg. Likewise Schlutz, Morse and Oldham fed an infant a daily dose of 100 mg. of iron in this form plus 0.75 mg. of copper and found a large iron retention though practically no hemoglobin regeneration, but here again the subject was markedly anemic. Certainly these are not conditions comparable to those of subject A who was receiving only a 5 mg. supplement of iron and who was, moreover, normal when the supplement was begun.

A possible explanation of the failure of subject A to store the additional iron given her orally is found in the result of the study by Heath et al. ('32) of hemoglobin regeneration following oral and parenteral administration of ferric ammonium citrate to patients with hypochromic anemia. He found that it required a daily dose of 1000 mg. of metallic iron when given orally as ferric ammonium citrate to produce the same blood-building effect as a daily dose of 32 mg. of metallic iron when given parenterally in the same form. That absorption of the iron in this form was the limiting factor appears likely though this is not in accord with the results of the studies on the availability of iron from ferric ammonium citrate.

The lack of correlation between the iron balances and the hemoglobin values for the different periods is not unexpected when the difference in the amount of iron required to make a significant change in these values is calculated. For subject A a change of 140 mg. in the amount of iron circulating in the blood stream would be required to vary the hemoglobin value 1 gm. per 100 cc., and then because of the method used for its determination such a change would be considered significant only if it resulted from many analyses over a period of several weeks. This change nevertheless involves ten times the amount of iron lost during a single menstrual period by this subject. On the other hand a change as small as 1 mg. in the average daily total retention of iron over a shorter period of time may

be considered significant in relation to the intake or excretion. It is not to be expected that iron retention and hemoglobin formation parallel each other for the retention of iron is but one step in its utilization.

A study of the complete metabolic picture for the subjects for the entire period is important for an understanding of their actual iron requirement. Following the current practice of considering the amount of a mineral which is excreted as representing the minimum requirement for that mineral, the minimum total daily iron requirement to cover the complete normal losses during a menstrual cycle for subjects A (omitting the periods of iron therapy), B, C and D, would be 11.9, 11.7, 9.16 and 11.9 mg., respectively. It is important that these minimum intakes would not provide for the replacement of any extra or unusual loss of iron such as a nosebleed or taking venous blood samples. Even though the subjects were receiving approximately only their minimal requirement of iron from the diet they increased the hemoglobin content of their blood slightly more than 1 gm. per 100 cc. This increase represented the utilization of 120 to 140 mg. of iron which had to come from bodily stores. Further increases in hemoglobin values would have been advisable but to what extent they would have been possible without replenishment of bodily stores is a question. Furthermore it is reasonable to assume that the maintenance of a level of iron stores above that of bare minimum is in greatest accord with optimum health. This together with the fact that iron is needed in the body for purposes other than blood building indicates the need of an intake of iron for these subjects which is greater than their minimal requirement. It has been customary to add 50% to a minimal requirement of a dietary essential and designate the result as an optimal allowance. On this basis the optimal allowance for subjects A, B, C and D would be 17.8, 17.5, 14.4 and 17.8 mg. of iron daily.

These results suggest certain facts regarding the iron requirement of normal women. The average daily fecal and urinary excretion of iron for all subjects for the entire time

of the study was 0.193 mg. per kilogram. Considering this figure as the minimal requirement and 50% above this or 0.289 mg. as the optimal allowance, the respective values for a 56 kilo. woman would be 10.8 mg. and 16.2 mg. The question then arises as to whether the optimal allowance will provide for the replacement of iron lost periodically in the menses or whether a separate allowance should be made to cover this loss. This will depend on two factors, the quantity of iron lost in the menses and the length of the menstrual cycle or the time between losses when the body can compensate for the previous loss or store iron in advance of the next one. Using Barer's average figure of 19.5 mg. for the iron content of the menses and assuming a menstrual cycle of 28 days in length it may be calculated that an average daily retention of 0.7 mg. would be necessary to compensate for such a menstrual loss. The dietary standard for a normal 56 kilo. woman would thus approximate 17 mg. per day. Another method of using the results of this study to calculate the daily iron requirement of women is to take the average total loss per kilogram per day of all the subjects, increase it 50% and multiply by the weight of an average woman. Thus 0.206 mg. per kilogram per day which covers losses in the feces, urine, and menses would be increased to 0.309, and then multiplied by 56 to give the total daily optimal allowance of iron which is 17.3 mg. The results obtained by either method of calculation are higher, though in reasonable agreement with the standard of 15 mg. of iron per day recommended by Sherman ('33).

It is a well-known fact that unless particular care is taken in planning a diet it will not contain the recommended 15 mg. of iron per day. The diet in the present study bears evidence to this. It was planned to represent a well-chosen mixed dietary with no emphasis on foods rich in iron and as a result it averaged only 10 to 13 mg. per day.

Parallel with the generally accepted intakes of iron which are lower than optimum are the generally accepted lower hemoglobin values for women. The latter are usually explained as due to the periodic loss of blood in the menses and

therefore were probably unavoidable. Since our present knowledge of the relatively small losses of iron and blood in the menses minimizes this as an etiologic factor it would seem that the low hemoglobin values are more directly a reflection of the prevalence of iron-deficient diets consumed over long periods of time than of excessive losses of iron in the menses. This does not mean that even a small loss should be disregarded when it is recurrent and continues through many years as does the menstrual loss, but neither should it be used to justify low hemoglobin values if they could be corrected by the ingestion of diets optimum in iron content.

SUMMARY AND CONCLUSIONS

The total iron exchanges of four normal young women on an adequate diet during consecutive menstrual cycles were determined over a period of 3 to 5 months by means of a continuous balance experiment.

The total time of the experiment was divided arbitrarily into consecutive 5-day periods and a single foundation dietary was used for every subject during every period. Certain low-mineral foods were permitted ad libitum to meet the differing energy requirements of the subjects. Food composites which represented one-tenth the amount of all food eaten by each subject were made and analyzed for each period.

Feces and urine collections were combined for each 5-day period and analyzed for iron. The menstrual flow was collected on cellulose pads for analysis. Records were kept of any additional blood losses and the results considered in the actual iron balance of the subjects.

The average daily intakes of iron for the entire time for the four subjects were 13.61, 11.87, 10.03 and 11.71 mg. with corresponding balances between food and excreta of + 0.72, + 1.48, + 0.71 and — 0.20 mg. The average coefficient of variation was 11.9 for the iron in the food and 25.8 for that in the excreta.

Although there were periods of negative and of positive balance for the subjects during the study, a negative balance never occurred when the intake of iron from food was 0.225

mg. or more per kilogram per day. No relation between time of iron storage and its loss during the menstrual period could be demonstrated.

A supplement of 5 mg. of iron in the form of ferric ammonium citrate was given to one subject during her fourth and fifth menstrual cycles. This resulted in an increased excretion and a decreased retention of iron as compared with the cycles when she was not receiving the supplement.

The average menstrual losses for the four subjects were 14.26, 22.84, 11.13 and 13.80 mg. of iron, respectively. The losses were relatively constant from period to period for the same subject.

In nine of the sixteen menstrual cycles the subjects were in iron equilibrium or slight positive balance after the menstrual loss had been subtracted from the balance between the iron intake and iron excretion for each cycle.

Only two subjects were able to compensate also for the iron loss in the venous blood samples which were taken in connection with another study. The other two subjects were in negative iron balance when this additional loss was included in calculating their actual iron balance for the entire study.

All the subjects increased the hemoglobin content of their blood at least 1 gm. per 100 cc. during the experimental regime; the red cell content remained remarkably constant.

The findings are discussed in relation to the iron requirement of these subjects and of other normal women. These subjects on a diet which contained 10 to 14 mg. of iron daily and fortified in other dietary essentials were receiving only their minimal requirement of this element. Calculations for the optimal daily allowance of iron for a 56 kilo. woman give results of 16 to 17 mg. which is somewhat higher than the 15 mg. recommended by Sherman. The results of this study together with the fact that the average dietary seldom contains even 15 mg. of iron indicate that the low hemoglobin values which are accepted as normal for women because of the drain due to menstruation may be a direct reflection of the use of diets habitually low in iron rather than due to the small periodic loss in the menses.

LITERATURE CITED

BARER, A. P. AND W. M. FOWLER 1936 The blood loss during normal menstruation. J. Am. Obstetrics and Gynecology, vol. 31, p. 979.

COONS, C. M. AND R. R. COONS 1935 Some effects of cod liver oil and wheat germ on the retention of iron, nitrogen, phosphorus, calcium and magnesium during human pregnancy. J. Nutrition, vol. 10, p. 289.

DAVIDSON, J. AND J. A. LECLERC 1936 The variation in the mineral content of vegetables. J. Nutrition, vol. 11, p. 55.

FARRAR, G. E. AND S. M. GOLDHAMER 1935 The iron requirement of the normal human adult. J. Nutrition, vol. 10, p. 241.

FOWLER, W. M. AND A. P. BARER 1935 Iron retention following use of ferric ammonium citrate in hypochromic anemia. J. Am. Med. Assn., vol. 104, p. 144.

HEATH, C. W., M. B. STRAUSS AND W. B. CASTLE 1932 Quantitative aspects of iron deficiency in hypochromic anemia. J. Clin. Invest., vol. 11, p. 1293.

LEVERTON, R. M. AND L. J. ROBERTS 1936 Hemoglobin and red cell content of the blood of normal women during successive menstrual cycles. J. Am. Med. Assn., vol. 106, p. 1459.

OHLSON, M. A. AND K. DAUM 1935 A study of the iron metabolism of normal women. J. Nutrition, vol. 9, p. 75.

SCHLUTZ, F. W., M. MORSE AND H. OLDHAM 1933 The influence of fruit and vegetable feeding upon the iron metabolism of the infant. J. Pediatrics, vol. III, p. 225.

SHERMAN, H. C. 1933 Chemistry of food and nutrition, p. 251. The Macmillan Co.

STEARNS, G. 1929 A rapid method for the preparation of fecal digests suitable for use in nitrogen and mineral analyses. J. Lab. and Clin. Med., vol. 14, p. 954.

STUGART, R. 1931 Determination of iron in milk and other biological materials. Ind. and Eng. Chem. Analytical Edition, vol. 3, p. 390.

TOSCANI, V. AND P. REZNIKOFF 1934 The iron content of foods used in a municipal hospital. J. Nutrition, vol. 7, p. 79.

VAHLTEICH, E. M., M. S. ROSE AND G. MACLEOD 1936 The effect of digestibility upon the availability of iron in whole wheat. J. Nutrition, vol. 11, p. 31.